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RESEARCH ARTICLE

# CONCORDANCE OF THE ANALYTICAL PERFORMANCE OF AUTOIMMUNE ANTIBODIES ON THE HOB BIOCLIA® 6500 AUTOMATED IMMUNOASSAY ANALYZER TO THE PHADIA® 250 SYSTEM

Knies C[1], Olivo M[2], Tönnies R[2], von Landenberg P[1]

[1] *LADR GmbH MVZ Nord-West, Adress: Technikerstr. 14, 48465 Schüttorf, Germany*
[2]*medac GmbH, Adress: Theaterstraße 6, 22880 Wedel, Germany*

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**ABSTRACT:** The prevalence of diagnosed autoimmune conditions in 2019 shows that the leading countries are the United States (7% of adult population), followed by the EU (6%) and China (5%). [1]There are more than 100different ADs (autoimmune diseases) and they are the third most common diseases worldwide. The difficulty in diagnosis, based on the clinical heterogeneity of the different types of AD and furthermorethe generated autoantibodies are often not specific for a single disease. In fact, there is a need to increase the clinical efficiency in the autoimmune diagnostic field. Therefore, we tested and compared the CLIA-test-based HOB BioCLIA 6500® in both, handling and performance,to the FEIA-based Phadia 250® system. 23 selected autoimmune parameters (e.g. in ANA, celiac disease or anti-phospholipids syndrome) and altogether 6401 measurements are done in our high-throughput lab. For the performance, the non-compliance and the κ–values are calculated to describe the effect of discrepant results. For 16 of 21 compared parameters we found a good compliance. Notwithstanding for some of the parameters, e.g. celiac and rheumatoid parameters, a discrepancy is observed. In an additional "celiac project" we bought characterized sera from the in.vent Diagnostica GmbH and repeated the measurement on the systems together with IFT on endomysium slides. In this second part of the study a very good agreement was found.

**KEYWORDS*:*** Chemiluminescentimmuno assay (CLIA); Automated autoimmune analyser; Autoimmune diseases (AD); HOB BioCLIA® 6500; Cohen's Kappa (κ) test; indirect fluorescence test (IFT); celiac disease.

## INTRODUCTION:

Autoimmune disorders are caused by an abnormally low or over activity of the immune system. For example, SLE (systemic lupus erythematosus) and RA (rheumatoid arthritis) are caused, as far as we know, by an over activity. With more than 100 ADs, they are the third most common diseases worldwide after cardiovascular disease and cancer. [2,3] The biggest challenge for diagnosis, is the non-specificity of the generated autoantibodies for a single disease, but their close relationship to clinical manifestations. [4]For the detection of some of those autoantibodies IFT is the gold standard. [5]However, the method requires intensive personal operation, evaluation and lacks sometimes reproducibility. [6]

**Corresponding Author:**
**Dr. Christine Knies,**
**LADR GmbH MVZ Nord-West, Adress: Technikerstr. 14, 48465 Schüttorf, Germany**
**Email-** Christine.Knies@labor-nordwest.de

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With regard to this background, there is a need for specific and sensitive tests to increase the clinical efficacy of antibody tests and to create easy running handling systems for diagnostic labs. Alternative methods for the detection of antibodies in the autoimmune field are EIAs.[1] One of the established systems on the European market using the EIA method is thePhadia250® system (ThermoFisher Scientific, Uppsala, Sweden). The aim of the present work was the practical application of the HOB BioCLIA® 6500 and the comparison to the Phadia® 250 system.

**Table 1: Comparison of the used systems.**

| | Phadia® 250 | HOB BioCLIA® 1200 | HOB BioCLIA® 6500 |
|---|---|---|---|
| **Sensitivity** | $>10^{-15}$mol/L | $10^{-18}$ mol/L | $10^{-18}$ mol/L |
| **Dynamic range** | $<10^5$ | $10^7$ | $10^7$ |
| **Method** | FEIA | CLIA | CLIA |
| **Through put** | 60 T/h | 60 T/h | 100-218 T/h |
| **Sample loading** | Random access | Random access | Random access |
| **Flexibility** | Flexible selection | Flexible selection | Flexible selection |
| **Reagents** | Stored on board | Stored on board | Stored on board |
| **Available Analytes** | 51 | 51 | 51 |

## MATERIALS AND METHODS:

### *HOB BioCLIA® 6500*

The HOB BioCLIA® 6500chemiluminescent immunoassay system utilizes streptavidin-coated magnetic nanoparticles (beads). The homo-tetramers of the protein have an extraordinarily high affinity for biotin ($K_d \approx 10^{-14}$mol/L) [7], e.g. the non-covalent interaction is one of the strongest in nature. [8] After the beads are incubated with the diluted serum and washed, antihuman IgG conjugate antibody as a tracer is added. The generated conjugate is enzymatically oxidized with an e.g. alkaline phosphatase solution (indirect CLIA) as trigger and chemiluminescent light is produced. [9]

The analytical performances including LOD (limit of detection), precision (including intra-assay and inter-assay), linearity and the effect of interfering substances were evaluated in accordance with relevant *Clinical & Laboratory Standards Institute* (CLSI) guidelines. [14]

For the concordance testing we used a selection of 23 parameters including antibodies for vasculitis, thyroid, anti-Phospholipids syndrome, celiac disease, rheumatoid arthritis and ANA.

### *Phadia® 250*

The Phadia® 250 system (ThermoFisher Scientific, Uppsala, Sweden) is a fully automated FEIA system for allergy and autoimmunity testing, designed as a sandwich immunoassay. [10,11].

It was used for the quantitative screening for the presence of autoantibodies. The used method in this system is an ELiA (FEIA), designed as a sandwich immunoassay. Quantitative detection of the antibodies in sera or plasma is done according to manufacturer's protocol. Calibrations are done with commercial standards in double determination every 28 days or after charge change of the conjugate. The evaluation of the results of the single control probe measurements is done according to the declared manufacturer areas for the quality management. In addition to the internal controls, periodic external quality controls via quality club (Phadia, monthly) and interlaboratory tests (RfB, two times per year) are done.

### *Statistical analysis*

For the statistical analysis Microsoft Excel 2010 for Windows was used. The "non-compliance" is calculated as the quotient of discrepant results to the total number of measurements per parameter. The degree of agreement of the antibody concentration determinations, and therefore for the positive-positive-, negative-negative- and the discrepancy-rate in the Phadia® 250 and HOB BioCLIA® 6500 system, the Cohen's kappa (κ) is calculated. [12]The statistical analysis is done for parameters with sample numbers >30. Thus, for the parameters anti-GBM (n= 6 sera), anti-CENP-B (n= 1 sera), there are no calculations done.

***Materials***

Patient sera with and without autoimmune disease were used for the study. In most cases clinical information or e.g. pregnancy were absent. Hence, we can't say which of the two systems is the preferable gold standard for an automated autoimmune analyser in relation to the medical conditions.

Patients were included with an age range of all ages, minimum a view week (0) and maximum 93 years. The main proportions are the age group 51-65 years followed by the 36-50 years old patients, as expected.

Overall, we had requirements from hospitals (about 15%) and outpatient clinics (85 %).

Calibrator and control reagents are supplied by the HOB Biotech Group and stored cold (4-8 °C). For calibration, high- and low- calibrators are used for a master curve principle, but not for anti-TPO and anti-TG. For each of those there were six calibrators, which built the master curve for the QC. As controls, a high and low positive control for each parameter was used. The HOB Biotech Group uses test-specific control materials, as requested from the RiliBÄK(The term '*RiliBÄK*' is an abbreviation meaning literally the Guidelines ("Rili") of the German Federal Medical Council (BÄK)). The patient panel was first tested at the Phadia 250® system, then at the BioCLIA 6500®.

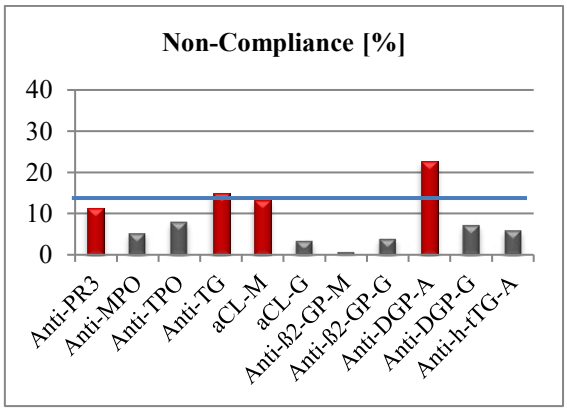
***Routine Procedure***

The cooled control and/or calibration materials are heated up at room temperature on the roll mixer for 20-25 min. Filling levels of sample tubes, wash buffer, waste etc., have to be checked. The prepared materials are inserted into the samples chamber and the barcode is scanned automatically. After pipetting, the controls and calibrators were unloaded from the sample racks and stored cold directly. After the run the patient samples are started.

## RESULTS:

*Comparison of the concordance: BioCLIA® 6500 versus Phadia® 250*

This study was conducted in the serological department at the LADR GmbH MVZ Nord-West in Schüttorf, Germany, a private lab for laboratory medicine. A comparative study of the HOB BioCLIA® 6500 (HOB Biotech Group, China) and the established Phadia® 250 system (ThermoFisher Scientific, Sweden) was performed. Between the beginning of December 2018 and the beginning of March 2019, altogether 6401 measurements of distributed on 23 parameters are done (see appendix, Table 03). Additionally, control measurements (RU/mL) at the BioCLIA® 6500 system are done and compared to the Phadia® 250 system on the same-day. Besides the comparison of the analyses, the operability and the daily handling is an additional point in the evaluation.The comparison resp. concordance of the parameters concerning the discrepancy results and the κ-values of the Cohen's kappa test are shown in graph 01 to graph 04.In terms of agreement between the different methods the Parameters anti-β2-GP-M (p= 0.8 %), anti-Jo-1 (p= 0 %), anti-RNP (p= 0.7 %) and anti-SS-B/La (p= 0.9 %) achieved the best agreements with a compliance >99.0 % for the detection of the antibodies in the sera.

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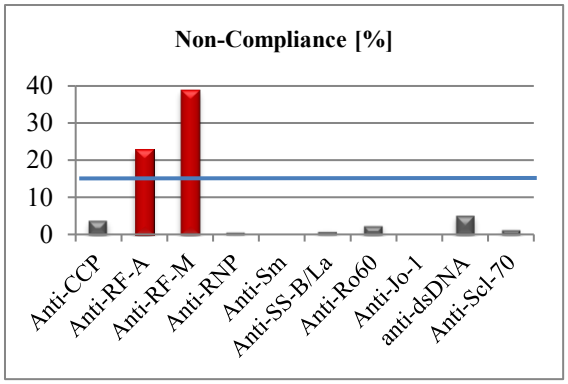



**Graph 1 and 2: Overview of the parameters and their non-compliance (positive-negative/negative-positive measurements) between the Phadia® 250 and HOB BioCLIA® 6500 system. Values of < 10 % are acceptable and grey coloured. Red coloured bars have a non-compliance of ≥ 10 % and show a bold difference. Minimum one positive-positive-result is measured expecting anti-Jo-1 and Sm.**

## ANCA

Anti-PR3 showed in contrast to the HOB BioCLIA® 1200 result (5.2 %, 135 sera) a non-compliance (11.4 %). A reason could be the slight number of samples from 44. Anti-MPO showed a good compliance again.

## ATA

The antithyroid antibodies showed no difference to the HOB BioCLIA® 1200 results. Anti-TPO is with a non-compliance of 7.9 % (before: 7.4 %) and anti-TG 15 % (before: 11.1 %).

## APL

For the antiphospholipid antibodies, anti-ß2-GP-M showed a lower non-compliance of 0.8 % (130 sera). The non-compliance on the HOB BioCLIA® 1200 was 6.5 % (just 31 sera).

## Celiac disease

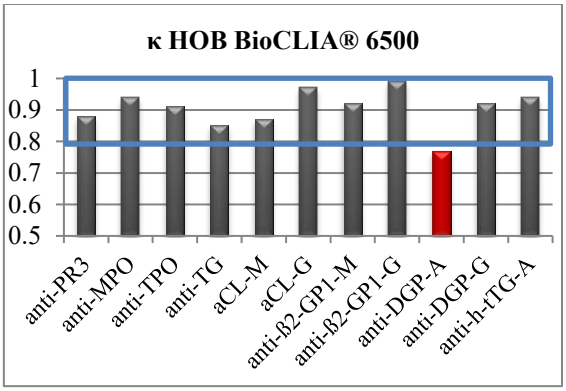
Noticeable is the constant non-compliance of anti-DGP-A with 22.6 % (HOB BioCLIA® 1200: 21.7 %). Anti-DGP-G and-h-tTG-A showed a good compliance, again. The repeated and extended study see "celiac project".

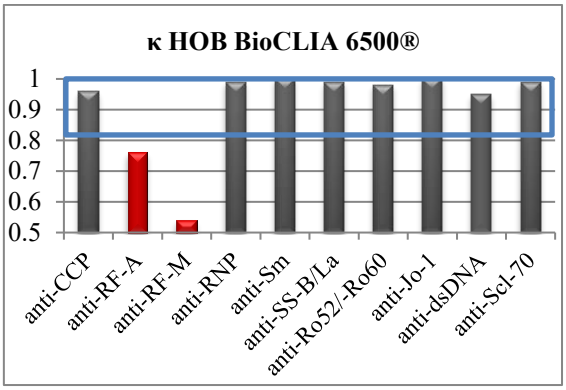
## RF

Anti-RF-A showed an increased non-compliance from 22.8 % (192 sera) starting from 6.3 % (31 sera) on the HOB BioCLIA® 1200. Anti-CCP and –RF-M showed similar non-compliances like the HOB BioCLIA® 1200. Anti-CCP 3.7 % (before: 5.2 %), anti-RF-M 38.9 % (before: 31.3 %). The repeated and extended study see "rheumatoid factors- project".

## ENA

Results between 0 and 2.3 % non-compliance are observed. The extractable nuclear antigens have constant results in comparison to the HOB BioCLIA® 1200.




**Graph 3 and 4: Calculated κ-values in the Cohen's kappa test. Strength of agreement: 1.00-0.81: almost perfect (blue box); 0.80-0.61: substantial; 0.60-0.41: moderate; 0.40-0.21: fair; 0.20-0.00: slight; <0.00 poor.** [13]

We excluded Anti-GBM and –CENP-B because of too small numbers of sera samples.

As calculated for the HOB BioCLIA® 1200, the calculated κ-values showed a reduced strength of

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agreement for the parameters anti-DGP-A and -RF-M and additionally just for the HOB BioCLIA® 6500 anti-RF-A. As a result, we did two further projects.

## Rheumatoid factors-project

The *first project* was the "rheumatoid factors-project". Therefore, we concordsera on three different systems: (a) the in-vitro-test for the quantitative determination of total RF with Roche/Hitachi cobas c701/702 system. The RF test from Roche® is an agglutination test with latex particles, the mixture of the different immunoglobulin types isn't known. (b) The ELiA RF-IgM and –IgA test from Thermo Fisher Scientific on the Phadia® 250 system and (c) the CLIA RF-IgM, -IgA and –IgG test on the HOB BioCLIA® 6500. The RF-IgG results aren't mention in fact of less importance.

We used the total RF (Roche) as reference, but no clinical data were available.

**Table 2: Positive-negative results in the systems with total RF as reference.**

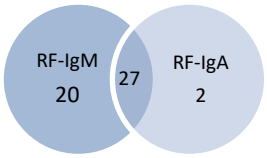
| Total RF (Roche) | RF-M Roche®/Phadia®/BioCLIA® | | RF-A Roche®/Phadia®/BioCLIA® | |
|---|---|---|---|---|
| **57 pos.** | ppp | 32 | ppp | 14 |
| | pnp | 15 | pnp | 15 |
| | ppn | 6 | ppn | 1 |
| | nnn | 4 | nnn | 27 |
| **18 neg.** | ppp | 12 | ppp | 17 |
| | pnp | 3 | pnp | 0 |
| | ppn | 3 | ppn | 1 |
| | nnn | 0 | nnn | 0 |

*Legend: ppp= pos./pos./pos.; pnp= pos./neg./pos.; ....; in the order Roche®/Phadia®/BioCLIA®.*

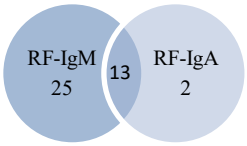
Total number of sera: 75, a complete concordance with no discrepant result to the Roche test (all results positive (ppp) or negative(nnn)) had 44 sera, a concordance with a discrepant result to the Roche test (example Roche® positive, Phadia® negative and BioCLIA® positive) had 4 sera.

In respect of the immunoglobulin types relating to a positive total RF, in the HOB system most of the observed results (n= 27) are positive in both, IgM and –A, followed by IgM (n= 20) and just 2 isolated IgA. In 9 cases a positive total RF but a negative HOB BioCLIA® result is observed.

**Graphic 1: Immunoglobulin types HOB BioCLIA® 6500anda positive total RF (Roche®).**




**Graphic 2: Immunoglobulin types Phadia® 250 and a positive total RF (Roche®).**




In contrast to the HOB BioCLIA® 6500, in the Phadia® system most of the observed results (n= 25) are positive in just IgM, followed by IgM plus IgA and last just two isolated IgA, like in the HOB system. In 18 cases a positive total RF but a negative Phadia® 250 result is observed.

In the setting of our rheumatoid factors-study the detailed characteristics of the patients are not known. Thus, the description of sensitivity and specificity of the assays could not be determined. To a future study with well characterized sera this will be done as well. In our current study only the performance data in comparison with the most widely used assay system was aim of our efforts.

## Celiac-project

The *second project* was the "celiac-project".

(a) Concordance of the Phadia® 250 and BioCLIA® 6500 with 117 additional sera from patients.
(b) Characterized sera from in.ventDiagnostica GmbH, Henningsdorf.

(a)

In the clinical diagnosis, anti-h-tTG-A is the most important parameter followed by anti-DGP-A. But in case of an immunoglobulin A lack, anti-DGP-G and –h-tTG-G is used.

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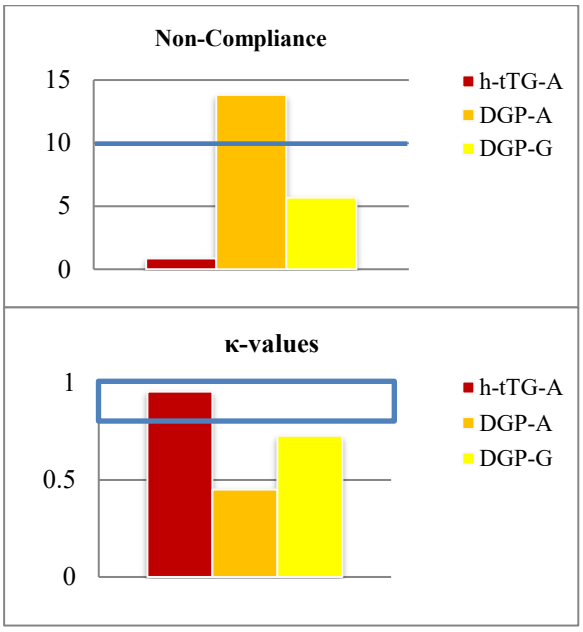
Calculated are the non-compliances and the κ-values for anti-h-tTG-A (n= 117), -DGP-A (n= 123), -DGP-G (n= 123) and –h-tTG-G (n= 13). Anti-h-tTG-G is in fact of too less samples not representative and therefore not shown.Few previous findings and clinical data were available. Most of the random samples were from women.




**Graph 5 and 6: Non-compliance of the celiac parameters (upper graph) and the calculatedκ-values in the Cohen's kappa test (downer graph). Strength of agreement: 1.00-0.81: almost perfect (blue box); 0.80-0.61: substantial; 0.60-0.41: moderate; 0.40-0.21: fair; 0.20-0.00: slight; <0.00 poor.** [13]

The humane tissue transglutaminase showed a very good compliance from 99% as well as an almost perfect κ-value from 0.955. Anti-DGP-A showed a significant non-compliance from 14% and just a moderate (0.450) κ-value. The anti-DGP-G showed a non-compliance from 6% and substantial (0.730) κ-value.

(b)

To clarify the question "Which instrument reflect better the medical outcome?", even for the anti-DGP, we bought 17 characterized sera from 6 celiac patients (all female) from in.vent, measured them on both instruments (see appendix table 03 to 06) and did IFT.Discrepant resultsare measured twice as a control. For the concordance, clinical and measured data are provided from in.vent. Exception Ptient 3993, no measured data were available, just the clinical outcome.

### *Anti-h-tTG-A and –G*

For the humane tissue transglutaminase a very good compliance, is shown, like in the in the "celiac project" for h-tTG-A before (see (a)). In each immunoglobulin class two patient sera didn't accord with the in.vent data but with the Phadia® 250 ones (see appendix table 04).

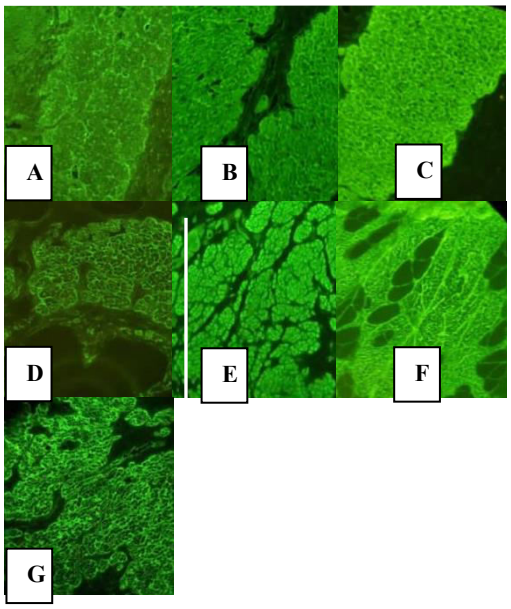
### *Anti-DGP-A*

For the deamidatedgliadin peptide, additionally, we did IFT as a control for the IgA class.For the detection of IgA antibodies against endomysium,a monkey oesophaguscommercial kit (NOVA Lite®; Inova Diagnostics, Inc., San Diego, USA) is used.The overview with the results is in the appendix, Table 05.

For just one patient (8422, sera 1-4, IFT picture F below) we observed a complete concordancefor all measurements. In the other four cases we got variety resultsfor one patient.

### Endomysium A IFT

**Graphic 3: Pictures of the Endomysium A IFT with the in.vent samples. Picture A-E and G: positive IFT test. Picture F: negative IFT. A+B: Patient 4548; C: Pat. 4578; D: Pat. 5051; E: Pat. 6371; F: Pat. 8422 and G: Pat. 3933.**



The concordance between the HOB BioCLIA® 6500 and the Phadia 250® system showed in 3 of 6 cases different results.

Nevertheless, the measured results at the BioCLIA® 6500 showed a perfect concordance to the IFTs.

***Anti-DGP-G***

Also, for anti-DGP-G measurements we observed in 3 cases different results (Patient 4548, 4578 and 6371). Patient 4548 and 4578 are negative characterized from in.vent, 6371 positive. For the patients 5051 and 8422 we observed the same results. Furthermore, a perfect concordance is observed between the BioCLIA® 6500 and Phadia 250® systems. See appendix, table 07.

## DISCUSSION:

Different immobilization techniques have led to advancement in the generation of immunoassays aimed to improve the specificity and sensitivity of the assays. But the aim of the diagnostic tests to distinguish between patients with and without an autoimmune disease is the same. ELISA tests are moderately fast with assay times between 1.5 to 3 hours. The focus shifted towards a decrease in assay time and fully automated technologies. To reduce time to result and minimize hands-on time in the laboratory new systems combining random access and CLIA technology have been developed and offer single patient testing together with assay times under 50 minutes. CLIAs are significantly different from ELISA techniques. As the antigen is covalently attached to the surface of the bead particles unlike the passive adsorption used for most ELISAs. [14]

Asin the HOB BioCLIA® 1200 system, we observedmore andhigher positive rates for the HOB BioCLIA6500® in comparison to the Phadia 250® system. Moreover, the celiac parameter anti-DGP-A and the rheumatic factor anti-RF-M had worse compliance and κ-values.

In the ENA-group, anti-DDS showed a non-compliance of 5% and a higher positive rate for the Phadia 250® system. Lower positive rates in

the HOB BioCLIA 6500® system in anti-DDS could be explained by the CLIA method´s wide dynamic range and therefore a higher analytical sensitivity. This observation suggests lower sensitivity and possibly higher specificity. Investigations demonstrated in a collaborative international study that anti-DDS serum concentrations measured by SLEDAI-2K and a CLIA instrumentation showed a strong correlation between them.[14]Nevertheless, very good concordances and k-values are observed between the two systems, as with the BioCLIA® 1200.According to the results of the marked EliA products test by ThermoFisher Scientific.[15] The specificity of these four parameters were observed with e.g. 97.9 % (anti-RNP) to 100 % (anti-Jo-1).[16]

As calculated for the HOB BioCLIA® 1200, the calculated κ-values showed a reduced strength of agreement for the parameters anti-DGP-A and -RF-M and additionally just for the HOB BioCLIA® 6500 anti-RF-A. As a result, we did two further projects.

The concord 75 sera on three different systems: (a) the in-vitro-test for the quantitative determination of total RF with Roche/Hitachi cobas c701/702 system; (b) The ELiA RF-IgM and –IgA test from Thermo Fisher Scientific on the Phadia® 250 system and (c) the CLIA RF-IgM, -IgA and –IgG test on the HOB BioCLIA® 6500. With these results we got information about the immunoglobulin classes, but no gold standard could nominate. A further project to the RF concerning clinical data is processed in a corporation with the AsklepiosFachkrankenhaus, Bad Abbach, Germany.

The third parameter group with a higher positive rate are the celiac parameters anti-DGP with the IgA- and IgG-classes. The higher positive rate seems not to be plausible, like at the BioCLIA® 1200. A point for discussion celiac was that the disease is not a relevant but rare disease in China. Thus, the assay could be far too sensitive for a Caucasian patient pool. [17] That's the reason why we decided to start two additional "celiac-projects": (a) The concordance of the Phadia®

250 and BioCLIA® 6500 with 117 additional sera from patients and (b) we bought

17characterized sera from 6 celiac patients from in.vent Diagnostica

GmbH, Henningsdorf, measured them on both system and in case of anti-DGP-A we did IFT, as a control.

In (a) anti-h-tTG-A showed a very good compliance as well as an almost perfect κ-value. Anti-DGP-A/-G showed substantial (0.73)/moderate (0.450) κ-values again.

For (b) to clarify the question "Which instrument reflect better the medical outcome?", most of all for the anti-DGP immunoglobulin classes, we got evidence, that the BioCLIA® 6500 observe good results for these parameters as related to the IFT.

Anti-PR3, showed at the BioCLIA® 6500 a non-compliance of 11.4 % with 44 sera (BioCLIA® 1200; 5.2%; 135 sera), in fact. The reason could be the slight number of samples. Anti-MPO showed a good compliance again.

One of the tasks of this study was to evaluate the daily routine in this high throughput lab. This is based mainly on subjective impressions of the technical personal being in charged for this study and well experienced in using fully automated instruments. The performance of the weekly and monthly service is very easy and not time-consuming. The software is neatly arranged, easy to handle and has anintuitive user interface/desktop. The system runs stable. Controls and calibrators have a bar code.

## CONCLUSION:

In conclusion, we evaluated the CLIA-based HOB BioCLIA 6500® in both, handling and test performance. We tested 23 parameters overall but calculated the statistical parameters only for 21 of these because of too less samples.For 16 of 21 compared parameters we found a good compliance. Notwithstanding, for some of the parameters, e.g. celiac parameters and the rheumatoid factors, a discrepancy to the Phadia® 250 system is shown. In the additional project with the in.vent-sera, we found a very good compliance for anti-h-tTG-A, anti-DGP-A and anti-h-tTG-G. The anti-DGP-G and IFT (Endomysium G) were discrepant, a reason could be the occurrence of much IgG in the tissue and therefore lower sensitivity in the IFT. The HOB BioCLIA® 6500 is a fast-processing instrument providing high throughput analysis for all necessary parameters.

## ABBREVIATIONS

CLIA: chemiluminescent immunoassay; FEIA: fluorescent enzyme immune assay; EIA: enzyme immune assay; resp.: respectively; κ: Cohen's Kappa; AD: autoimmune disease; IgG/A/M: immunoglobuline G/A/M; uv: ultraviolet; IFM: immunofluorescence microscopy; RfB: ReferenzinstitutfürBioanalytik; RU/ml: relative units per milliliter; $k_d$: dissociation constant; e.g.: exempli gratia or for example; ANA: antinuclear antibody; IFT: Immunofluorescence test; Pat.: Patient.

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**APPENDIX**

**Table 3: Overview of the non-compliances and statistical data, in detail. Red coloured data show a bad non-compliance (≥10 %). Values in brackets are the results from the HOB BioCLIA® 1200.**

| Parameter | Sera [n] | Non-Compliance [%] HOB BioCLIA® 6500 | κ HOB BioCLIA® 6500 |
|---|---|---|---|
| **Anti-PR3** | (135) 44 | (5.2) 11.4 | (0.94) 0.88 |
| **Anti-MPO** | (80) 77 | (5.0) 5.2 | (0.95) 0.94 |
| **Anti-TPO** | (866) 379 | (7.4) 7.9 | (0.92) 0.91 |
| **Anti-TG** | (208) 515 | (11.1) 15 | (0.89) 0.85 |
| **aCL-M** | (206) 150 | (11.7) 13.3 | (0.88) 0.87 |
| **aCL-G** | (199) 148 | (6.5) 3.4 | (0.93) 0.97 |
| **Anti-ß2-GP-M** | (31) 130 | (6.5) 0.8 | (0.94) 0.92 |
| **Anti-ß2-GP-G** | (26) 130 | (3.9) 3.9 | (0.96) 0.99 |
| **Anti-DGP-A** | (253) 527 | (21.7) 22.6 | (0.78) 0.77 |
| **Anti-DGP-G** | (235) 523 | (4.3) 7.2 | (0.96) 0.92 |
| **Anti-h-tTG-A** | (714) 814 | (3.8) 2.9 | (0.96) 0.94 |
| **Anti-CCP** | (1010) 1469 | (5.2) 3.7 | (0.95) 0.96 |
| **Anti-RF-A** | (127) 192 | (6.3) 22.8 | (0.94) 0.76 |
| **Anti-RF-M** | (131) 191 | (31.3) 38.9 | (0.68) 0.54 |
| **Anti-RNP** | (231) 145 | (1.3) 0.7 | (0.99) 0.99 |
| **Anti-Sm** | (205) 48 | (2.0) 0 | (0.98) 1 |
| **Anti-SS-B/La** | (211) 212 | (1.4) 0.9 | (0.99) 0.99 |
| **Anti-Ro52/-Ro60** | (413) 215 | (3.8) 2.3 | (0.96) 0.98 |
| **Anti-Jo-1** | (187) 54 | (1.1) 0 | (0.99) 1 |
| **dsDNA** | (305) 278 | (8.5) 5 | (0.91) 0.95 |
| **Anti-Scl-70** | (198) 153 | (1.0) 1.3 | (0.99) 0.99 |
| **Anti-GBM** | (7) 6 | - | - |
| **Anti-CENP-B** | (4) 1 | - | - |

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**Table 4: Anti-h-tTG-A and –Gdata from the in.vent sera, measured on the two instruments. The data from in.vent are given by them. CO: control measurement (a repetition is done). Red coloured lines show a positive, green coloured lines a negative result.**

| | | in.vent | BioCLIA® 6500 | Phadia® 250 |
|---|---|---|---|---|
| **Pat.-ID (in.vent)** | Sample | h-tTG-A | h-tTG-A | h-tTG-A |
| **4548** | 1 | ELISA 27,3 | 31,6 | 15,9 |
| | 2 | | 26,39 | 12,7 |
| | 3 | | 27,72 | 12,4 |
| | 4 | | 27,75 | 13,1 |
| | 5 | | 28,94 | 14,7 |
| | 6 | | 26,84 | 12,9 |
| | 7 | | 22,04 | 11,6 |
| | 8 | | 27,59 | 12,1 |
| | 9 | | 23,95 | 11,8 |
| **4578** | 1 | ELISA 15,8 | 22,71 / CO: 26,41 | 12,8 / CO: 14,8 |
| **5051** | 1 | ELISA 6,39 | 6,67 | 4,3 |
| **6371** | 1 | ELISA (Phadia)35 | 2,67 / CO: 2 | 0,9 / CO 1,0 |
| **8422** | 1 | ELISA (Phadia) 17 | 21,18 | 15 |
| | 2 | | 22,08 | 14,5 |
| | 3 | | 22,77 | 15,2 |
| | 4 | | 21,48 | 13,8 |
| **3993** | 1 | ELISA 767 in.vent | 185,59 BioCLIA® 6500 | 142 Phadia® 250 |
| **Pat.-ID (in.vent)** | Sample | h-tTG-G | h-tTG-G | h-tTG-G |
| **4548** | 1 | ELISA 22,3 | 3,54 | 2,5 |
| | 2 | | 3,51 | 2,3 |
| | 3 | | 3,69 | 2,2 |
| | 4 | | 3,75 | 2,4 |
| | 5 | | 4,11 | 2,4 |
| | 6 | | 3,44 | 2,6 |
| | 7 | | 2,92 | 2,4 |
| | 8 | | 3,36 | 2,4 |
| | 9 | | 3,27 | 2,3 |
| **4578** | 1 | ELISA 57,8 | 3,25 | 2,2 |
| **5051** | 1 | ELISA 0 | 2,35 | 2,2 |
| **6371** | 1 | ELISA (Phadia) 0,4 | 2 | 1,3 |
| **8422** | 1 | ELISA (Phadia) 0,35 | 2 | 1,6 |
| | 2 | | 2 | 1,5 |
| | 3 | | 2 | 1,5 |
| | 4 | | 2 | 1,4 |
| **3993** | 1 | ELISA 100 | 4,85 | 6,3 |

**Table 5: Anti-DGP-A data from the in.vent sera, measured on the two instruments and results from the Endomysium An IFT. The data from in.vent are given by them. CO: control measurement (a repetition is done). Red coloured lines show a positive, green coloured lines a negative result.**

| | | in.vent | BioCLIA® 6500 | Phadia® 250 | Endomy. A | Endomy. A (CO) |
|---|---|---|---|---|---|---|
| **Pat.-ID (in.vent)** | Sample | DGP-A | DGP-A | DGP-A | Screen / 1:40 | Screen |
| **4548** | 1 | neg. | 50.6 | 4 | pos./pos. | pos. |
| | 2 | | 43.4 | 3.6 | - | |
| | 3 | | 42.44 | 3.3 | - | |
| | 4 | | 31.81 | 3.5 | - | |
| | 5 | | 46.94 | 3.9 | - | |
| | 6 | | 39.32 | 3.5 | - | pos. |
| | 7 | | 41.84 | 3.2 | - | |
| | 8 | | 41.97 | 3.5 | - | |
| | 9 | | 32.77 | 3.5 | - | |
| **4578** | 1 | neg. | 175.6 / CO: 167.4 | 14.1 / CO: 16.4 | - | pos. |
| **5051** | 1 | neg. | 74.37 | 4.7 | - | pos. |
| **6371** | 1 | ELISA (Phadia) 25 | 4.7 / CO: 4.53 | 0.7/ CO: 0.6 | neg./neg. | neg. |
| **8422** | 1 | ELISA (Phadia) 81 | 400 | 165 | pos./pos. | |
| | 2 | | 400 | >142 | - | |
| | 3 | | 400 | 151 | - | |
| | 4 | | 400 | 165 | - | |
| **3993** | 1 | - | 32.89 | 3.2 | pos./pos. | pos. |

**Table 6: Anti-DGP-G data from the in.vent sera, measured on the two. The data from in.vent are given by them. CO: control measurement (a repetition is done). Red coloured lines show a positive, green coloured lines a negative result.**

| | | in.vent | BioCLIA® 6500 | Phadia® 250 |
|---|---|---|---|---|
| **Pat.-ID (in.vent)** | Sample | DGP-G | DGP-G | DGP-G |
| **4548** | 1 | neg. | 61.52 | 14.8 |
| | 2 | | 56.61 | 11.9 |
| | 3 | | 55.33 | 12.7 |
| | 4 | | 51.07 | 12.3 |
| | 5 | | 64.58 | 14.2 |
| | 6 | | 51.38 | 12 |
| | 7 | | 55.85 | 11.6 |
| | 8 | | 54.86 | 12.5 |
| | 9 | | 48.34 | 10.6 |
| **4578** | 1 | neg. | 400 | 45.8 / CO: 49.9 |
| **5051** | 1 | pos. | 50.69 | 18.6 |
| **6371** | 1 | ELISA (Phadia) 82 | 2.24 / CO: 2.04 | 0.4 |
| **8422** | 1 | ELISA (Phadia) 31 | 400 | 39.6 |
| | 2 | | 400 | 42.6 |
| | 3 | | 400 | 35.3 |
| | 4 | | 400 | 35.2 |
| **3993** | 1 | - | 269.06 | 30.4 |

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